Study of Cytoprotective Effects of Lycopene on Azathioprine Induced Hepatic Injury in Rat

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Abstract: Azathioprine is a cytotoxic agent that is registered in the Netherlands since 1968 for use as immunosuppressant both after transplantation as in autoimmune or chronic inflammatory diseases. The aim of this study was to investigation of cytoprotective effects of lycopene on azathioprine induced hepatic injury in rats. In this study 30 wistar rats were allocated into the 3 groups of 10 rats. After six days, rats were euthanized and their liver was achieved to pathologic studies. Results showed that AST and ALT serum levels and hepatic injury in that group received Azathioprine were higher than control group and ALT serum level in that group received lycopene plus Azathioprine was more than control group and was less than group 2. In this study revealed that lycopene can alleviate hepatotoxicity effect of azathioprine but cannot be reach to the normal range.

Key words: cytoprotection, lycopene, azathioprine, hepatic injury, rats.

INTRODUCTION

Many reports from the epidemiologic literature infer that high intakes of tomato and tomato products are beneficial to health (Rao, A.V. and S. Agarwal, 1998). The benefits of such foods are attributed to their antioxidant properties, especially to the antioxidant properties of lycopene contained therein. In fact, antioxidant properties of lycopene have been investigated extensively as a potential protective agent against the cancers of the prostate, cervix, colon, breast, and other chronic diseases (Giovannucci, E., 1995).

Azathioprine (Imuran®) is a cytotoxic agent (Rang, H.P., 2012) that is registered in the Netherlands since 1968 for use as immunosuppressant both after transplantation as in autoimmune or chronic inflammatory diseases. It is used as monotherapy but often in combination with other medication (usually corticosteroids) and methods influencing immune response for rheumatoid arthritis, pemphigus vulgaris, chronic refractory idiopathic thrombocytopenic purpura, systemic lupus erythematoses, M. Crohn and colitis ulcerosa. Its use usually in combination with corticosteroids and/or other interventions is indicated for dermatomyositis, polymyositis, polyarteritis nodaso, chronic active hepatitis and haemolytic anaemia (auto-immune basis). Commonly occurring ADRs with azathioprine are gastrointestinal symptoms, hypersensitivity reactions, depression of bone marrow, immunosuppression related infection and hepatotoxicity (Dutch SmPC Imuran, 2006; Jarrett, P., 1997; Rang, H.P., 2012). Azathioprine’s mechanism of action remains to be elucidated, but suggested mechanisms for its immunosuppressive properties include:
- The release of 6-mercaptopurine (6-MP) which act as a purine antimetabolite
- The possible blockade of –SH groups by alkylation
- The inhibition of many pathways in nucleic acid biosynthesis, hence preventing proliferation of cells involved in determination and amplification of the immune response
- Damage to deoxyribonucleic acid (DNA) through incorporation of purine thioanalogues.

Azathioprine and long-wave ultraviolet light have been shown to have a synergistic clastogenic effect in patients treated with azathioprine for a range of disorders. As is usual for patients with an increased risk of skin cancer exposure to sunlight and UV light should be limited and patients should wear protective clothing and use sunscreen with a high protection factor. Photo-absorbing properties of azathioprine are described in literature (Jarrett, P., 1997; Karran, P., Thiopurines, 2006; O'Donovan, P., 2005). However, no publications are presented in a PubMed search using MESH terms azathioprine and phototoxic dermatitis. Nor were cases of immunologically mediated photosensitivity reactions published.

MATERIALS AND METHODS

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In this study, 30 wistar rats were allocated into the 3 groups of 10 rats. Group 1, as control group received normal saline. Group 2 received Azathioprine at the dose of 50 mg/kg (Hesham A. El-Beshbishy, 2010) as ip. Group 3 beside of Azathioprine received lycopene at the dose of 4 mg/kg (Fad Ayan, 2011) as oral. This group, 3 days before administration of the Azathioprine has received lycopene. After six days, rats were euthanized and their liver was achieved to pathologic studies. Also, serum samples to measurement of AST and ALT were obtained. The Statistical Package for Social Sciences (SPSS Inc., Chicago, IL, USA), version 13.0, was used for statistical analysis. All data are presented as mean ± SEM. Before statistical analysis, all variables were checked for normality and homogeneity of variance by using the Kolmogorov-Smirnoff and Levene tests, respectively. The data obtained were tested by ANOVA followed by Tukey's post-hoc multiple comparison test. P<0.05 was considered statistically significant.

**Results:**

Results showed that AST and ALT serum levels and hepatic injury in that group received Azathioprine were higher than control group and ALT serum level in that group received lycopene plus Azathioprine was more than control group and was less than group 2 (p<0.05). Table 1.

<table>
<thead>
<tr>
<th>Groups</th>
<th>ALT mg/dl</th>
</tr>
</thead>
<tbody>
<tr>
<td>1: control</td>
<td>27.00±4.83</td>
</tr>
<tr>
<td>2: Azathioprine only</td>
<td>120.25±12.36</td>
</tr>
<tr>
<td>3: Azathioprine plus lycopene</td>
<td>85.76±8.15</td>
</tr>
</tbody>
</table>

ALT: Alanine Amino Transferase

![Graph 1](image)

**Graph 1:** The comparison amount of ALT between control group, Azathioprine group, Azathioprine + lycopene group. Results are expressed as Mean±SE. *** p<0.001 significantly different from the control group.

AST serum value in that group received Azathioprine was higher than control group and AST serum value in group 3 was more than control group and was less than group 2 (p<0.05). Table 2.

<table>
<thead>
<tr>
<th>Groups</th>
<th>AST mg/dl</th>
</tr>
</thead>
<tbody>
<tr>
<td>1: control</td>
<td>28.60±7.41</td>
</tr>
<tr>
<td>2: Azathioprine only</td>
<td>141.50±6.34</td>
</tr>
<tr>
<td>3: Azathioprine plus lycopene</td>
<td>93.24±1.48</td>
</tr>
</tbody>
</table>

AST: Aspartate Amino Transferase

Finally, results showed that hepatic injury in group 2 was more than control group and in that group received Azathioprine + lycopene however was more than the group had not received Azathioprine but was less than the group that received Azathioprine alone (p<0.05). Table 3.
Graph 2: The comparison amount of AST between control group, Azathioprine group, Azathioprine + lycopene group. Results are expressed as Mean±SE. *** p<0.001 significantly different from the control group.

Graph 3: The comparison rate of hepatic injury between control group, Azathioprine group, Azathioprine + lycopene group. Results are expressed as Mean±SE. *** p<0.001 significantly different from the control group

Table 3: Hepatic injury index in groups at the end of the experiment.

<table>
<thead>
<tr>
<th>Groups</th>
<th>hepatic injury index</th>
</tr>
</thead>
<tbody>
<tr>
<td>1: control</td>
<td>0.30±0.48</td>
</tr>
<tr>
<td>2: Azathioprine only</td>
<td>11.30±2.11</td>
</tr>
<tr>
<td>3: Azathioprine plus lycopene</td>
<td>7.95±1.88</td>
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</tbody>
</table>

Discussion:
Azathioprine can be involved in oxidative stress both by increasing absorption of ultraviolet radiation and by diminishing reductive reactions by inhibiting niacin metabolism. A potential confounder can be inflammatory bowel disease activity since this can lead to reduction in intestinal niacin absorption. Yet two of nine patients with photosensitivity used azathioprine for rheumatoid arthritis and in the remaining patients no signs of increased disease activity, like extensive diarrhoea or intestinal surgery were mentioned.

First, the active substance 6-TG has an absorbance maximum of 342 nm wavelength which is within the UVA spectrum, giving this substance a potential for formation of free radicals or reactive oxygen species. In an in-vitro study photosensitivity effects of cellular damage, notably at DNA level, was demonstrated in cellular cultures that were exposed to low intensity UVA (O'Donovan, P., 2005). Furthermore dermal lesions related to sunlight exposure have been described both in relation to use of azathioprine for inflammatory bowel disease and in relation to the disease itself (Jarrett, P., 1997; Zaki, I., L. Millard, 1995). Symptoms resemble dermal lesions in pellagra, a disorder characterized by a deficiency in niacin (vitamin B3), due to dietary unbalances, or to malabsorption or to ingestion of substances blocking niacin metabolism (Zaki, I., L. Millard, 1995). 6-MP is implicated in blocking this pathway, and thus increases the chance for dermal lesions during sunlight exposure in inflammatory bowel disease (Jarrett, P., 1997).
Antioxidant Properties of Lycopene:

(i) Superoxide anion. Superoxide anions are important oxygen radicals that can occur during in vivo metabolism. But if in vivo superoxide anions are present in a higher than allowable concentration, they will destroy macromolecules like protein, DNA, and the like (Cheng, X.S., 1999). Thus, it is beneficial to explore the effect of LC on superoxide anions.

(ii) Hydroxyl radical. Hydroxyl radicals are the most aggressive oxygen species in vivo (Halliwell, B., 1995), and they are the most important killer for biomolecules.

(iii) Lipid peroxidation. Lipid peroxidation (Woodall, A.A., 1997) is a common product of oxidative stress in biological tissues such as lipoproteins, liposomes, microsomes, and membranes. The ability to reduce lipid peroxidation (Marklund, S. and G. Marklund, 1974; Woodall, A.A., 1997) has become an important factor in examining the biological benefits of antioxidants.

(iv) Singlet oxygen. Singlet oxygen (\(1^\text{O}_2\)) (Mortensen, A., 1997; Clinton, S.K., Lycopene, 1998), although not technically a free radical, is a very reactive high-energy and short-lived oxygen species that can react with biomolecules.

REFERENCES


